

REMARKS

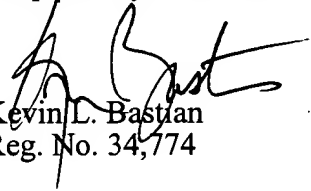
In accordance with 37 C.F.R. §§1.821 to 1.825, Applicants request entry of this amendment. This amendment is accompanied by a floppy disk containing SEQ ID NOS:1-4, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

  
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 15 of page 22 has been amended as follows:

Due to the basal regulation of anion and  $K^+$  channels in *abh1* without addition of exogenous ABA, experiments were pursued to analyze whether mechanisms lying further upstream confer ABA hypersensitivity in *abh1*. Anion channels are activated and inward-rectifying  $K^+$  channels are down-regulated by upstream  $[Ca^{2+}]_{cyt}$  elevations (J.I. Schroeder & S. Hagiwara, *Nature*, 338:427 (1989)). Therefore we directly investigated whether *abh1* modulates ABA-induced  $[Ca^{2+}]_{cyt}$  elevations in time-resolved cameleon  $[Ca^{2+}]_{cyt}$  imaging experiments (G. J. Allen *et al.*, *The Plant J.*, 19:735 (1999)). Stomata were opened by exposing plants for 12 hours to 95% humidity. In wild-type, 56 % (n=32 of 57) of guard cells showed no  $[Ca^{2+}]_{cyt}$  increase in response to a low concentration of 0.5  $\mu M$  ABA and the remaining 44% (n=25) cells typically showed only one  $[Ca^{2+}]_{cyt}$  increase with an average peak increase of  $170 \pm 25$  nM  $[Ca^{2+}]_{cyt}$  (~~Fig. 2D, bottom~~). Interestingly, in *abh1* guard cells, 0.5  $\mu M$  ABA elicited  $[Ca^{2+}]_{cyt}$  increases in 64% of guard cells (n= 41 of 64 cells) with a larger average peak increase of  $280 \pm 22$   $\mu M$  (~~Fig. 2E, bottom~~). Only 19% of the cells (n=12) responded with one  $[Ca^{2+}]_{cyt}$  elevation while 45% of *abh1* cells (n=29) showed multiple repetitive  $[Ca^{2+}]_{cyt}$  increases at 0.5  $\mu M$  ABA (~~Fig. 2E, bottom~~). Only 36% of *abh1* cells (n=23) showed no response to 0.5  $\mu M$  ABA. Statistical analyses of responsive versus non-responsive cells confirmed that the ABA responsiveness of *abh1* guard cells was significantly enhanced ( $\chi^2=4.96$ ,  $P<0.03$ ). Furthermore both the number of  $[Ca^{2+}]_{cyt}$  transients per cell ( $P<0.001$ ) and their amplitudes ( $P<0.01$ ) were significantly larger in *abh1* than in wild-type.  $[Ca^{2+}]_{cyt}$  imaging analyses (~~Fig. 2D and E~~) and stomatal aperture measurements demonstrate that

the *abh1* mutation enhances early ABA signaling mechanisms upstream of ABA-induced  $[Ca^{2+}]_{cyt}$  elevations.

Paragraph beginning at line 17 of page 23 has been amended as follows:

The *ABH1* gene was identified by plasmid rescue and the corresponding cDNA (2547 bp) was isolated. Briefly, a 278 bp genomic fragment adjacent to the right border of the T-DNA insertion was isolated from *abh1* plants using plasmid rescue as follows: 5 µg of genomic DNA was digested with *Hind*III, self-ligated and transformed into *E. coli* ElectroMAX DH12S (GibcoBRL, Lifetechnology). Plasmid extracted from cells growing on carbenicillin was sequenced. Primers were then generated to amplify 5316 bp genomic DNA flanking the rescued sequence (GenomeWalker Kit, Clontech). A 8248bp *Cla*I genomic fragment containing the full *ABH1* locus was cloned from BAC T10F2 (*Arabidopsis* Biological Research Center) into the plant expression vector pRD400. *ABH1* coding sequences were amplified from an *Arabidopsis* Columbia leaf cDNA library by rapid amplification of cDNA ends (RACE PCR, Marathon cDNA Amplification Kit, Clontech) using the plasmid rescue sequence internal primer (5' GAAGCTCAACTCGTTGCTGGAAAG 3'; SEQ ID NO:4) and its reverse. The total cDNA of 2547 bp was then amplified using *pfu* DNA polymerase (Stratagene), cloned in pMON530 and sequenced. *ABH1* 5' UTR (1250bp) was amplified from genomic DNA by PCR using *pfu* DNA polymerase and subcloned in pCAMBIA1303 (Genbank AF23299) containing a promoterless glucuronidase reporter gene. All sequences amplified by PCR were checked by sequencing (Retrogen, CA).